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# Prevalence and Molecular Characterization of Pertactin-Deficient *Bordetella pertussis* in the United States

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**Pertussis has shown a striking resurgence in the United States, with a return to record numbers of reported cases as last observed in the 1950s. *Bordetella pertussis* isolates lacking pertactin, a key antigen component of the acellular pertussis vaccine, have been observed, suggesting that *B. pertussis* is losing pertactin in response to vaccine immunity. Screening of 1,300 isolates from outbreak and surveillance studies (historical isolates collected from 1935 up to 2009, isolates from the 2010 California pertussis outbreak, U.S. isolates from routine surveillance between 2010-2012, and isolates from the 2012 Washington pertussis outbreak) by conventional PCR and later by Western blotting and *prn* sequencing analyses ultimately identified 306 pertactin-deficient isolates. Of these pertactin-deficient strains, 276 were identified as having an IS*481* in the *prn* gene (*prn*IS*481* positive). The first *prn*IS*481*-positive isolate was found in 1994, and the next *prn*IS*481*-positive isolates were not detected until 2010. The prevalence of pertactin-deficient isolates increased substantially to more than 50% of collected isolates in 2012. Sequence analysis of pertactin-deficient isolates revealed various types of mutations in the *prn* gene, including two deletions, single nucleotide substitutions resulting in a stop codon, an inversion in the promoter, and a single nucleotide insertion resulting in a frameshift mutation. All but one mutation type were found in *prn*2 alleles. CDC 013 was a predominant pulsed-field gel electrophoresis (PFGE) profile in the pertactin-positive isolates (203/994) but was found in only 5% (16/306) of the pertactin-deficient isolates. Interestingly, PFGE profiles CDC 002 and CDC 237 represented 55% (167/306) of the identified pertactin-deficient isolates. These results indicate that there has been a recent dramatic increase in pertactin-deficient *B. pertussis* isolates throughout the United States.**

Reported pertussis cases in the United States have increased in the last 10 years with recent peaks in both 2010 and 2012 despite a successful vaccination program (1, 2). Record numbers of more than 48,000 cases were reported in 2012 (1). This resurgence has been attributed to many factors, such as improved surveillance capacity, increased awareness among clinicians and the public, more sensitive and specific laboratory diagnostics, and waning protection from the acellular vaccines (3, 4). Increased pertussis case counts have been seen in multiple countries with various vaccination programs (5–7). In the United States, the whole-cell pertussis vaccine was introduced for children in the 1950s and later replaced by an acellular vaccine in the 1990s. The purified protein components of the two major childhood acellular pertussis vaccines licensed in the United States both contain pertussis toxin, pertactin, and filamentous hemagglutinin (GlaxoSmithKline, Rixensart, Belgium, and Sanofi Pasteur, Lyon, France). One vaccine also contains fimbrial proteins 2/3 (Sanofi Pasteur).

The genomes of the currently circulating *Bordetella pertussis* isolates differ from the isolates used in the manufacture of vaccine components (8–12). Allelic variations in several of the acellular vaccine components, such as *ptxP3*, *prn2*, and *fim3B*, have appeared, and the predominant profile observed no longer completely matches the strains used to make the acellular vaccine components (12–15). Another genomic change has been the deletion of pertactin, a key antigen component of pertussis vaccines. *B. pertussis* isolates that lack the presence of pertactin protein, here called pertactin-deficient isolates, have been identified in France, Italy, Japan, and Finland (13, 16–19). Prevalences between countries have ranged from 2.6% in Finland (16) to 27% in Japan (19). In the United States, 11 pertactin-deficient isolates were recently reported in a collection of 12 contemporary isolates from a Philadelphia hospital (20). Overall, the mutations causing the pertactin deficiency vary widely and include IS*481* insertions, deletion of the 5′ signal sequence, premature stop codons, and a large deletion of the promoter and 5′ coding region (13, 16–20). To our knowledge, no longitudinal studies to interpret the progression of this deficiency over time have been described.

To better understand the timing of the emergence and prevalence of this phenomenon in the United States, we analyzed 1,300 isolates from the Centers for Disease Control and Prevention (CDC) collection that represent four different origins and time periods: historical isolates collected from 1935 up to 2009 (12), isolated from the 2010 California pertussis outbreak (21), U.S. isolates collected during routine surveillance between 2010 and 2012 from various state public health laboratories, including the Enhanced Pertussis Surveillance (EPS) system of the Emerging Infections Program (EIP) network, and isolates from the 2012 Washington pertussis outbreak (2). We found the emergence of pertactin-deficient isolates to be a very recent occurrence, their prevalence to be wide reaching, and the mutations creating the pertactin deficiency to be highly variable. This is the first report of

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**TABLE 1** Primers used for PCR screening and sequence analysis

| Primer name | Sequence (5′ to 3′) | Position on Tohama I relative to *prn* start codon[a] | Reference no. or source |
|---|---|---|---|
| PF | TGTCTCTGTCACGCATTGTC | 8 to 27 | 22 |
| PR | ATGCCGTTGGTGTGTACCGT | 2,551 to 2,570 | 22 |
| AF | GCCAATGTCACGGTCCAA | 505 to 522 | 22 |
| AR | GCAAGGTGATCGACAGGG | 1,073 to 1,090 | 22 |
| BF | AGCTGGGCGGTTCAAGGT | 1,398 to 1,415 | 22 |
| BR | CGGATTCAGGCGCAACTC | 1,915 to 1,932 | 22 |
| PRN-F | CGTACTTTTGCTGCGCCCAT | −79 to −60 | 19 |
| PRN-R | CCAAGCTCCAGGAAAACCTC | 2,761 to 2,780 | 19 |
| PRNUP2354 | GAGAGCCATTTACTGGAGATT | −2,353 to −2,333 | This study |
| PRN-P-F | TGCCAAGACGGTATCTGT | −331 to −314 | This study |
| PRN-P-R | GACTGGTTGTTCCAGTCG | 102 to 119 | This study |
| PRN342R | GGTGACGGTGCCCAGAAAGC | 323 to 342 | This study |
| PRNA2F | AGGGTGACGGTGTCGGGC | 982 to 999 | This study |
| PRN1627R | TATCGACCTTGCCGTCCTT | 1,627 to 1,645 | This study |
| PRNB2F | CAGCAGCTGGACAACCGC | 1,972 to 1,989 | This study |
| PRNP2R | CTTGCCCTTGACCGCGT | 2,258 to 2,274 | This study |
| PRN2258F | CGGTCAAGGGCAAGTACC | 2,261 to 2,278 | This study |
| PRN2357F | CCGAGCTGGCGGTATTC | 2,357 to 2,373 | This study |

[a] *prn* lies in the region between nt 1,098,091 and 1,100,823 of the Tohama I complete genome (GenBank accession no. NC_002929.2).

the recent and rapid expansion of pertactin-deficient isolates in the United States.

## MATERIALS AND METHODS

**Isolate collection.** The CDC collection bank included 1,300 *B. pertussis* isolates divided in four groups: (i) 666 historical isolates collected from 1935 up to 2009 (12), (ii) 33 isolates from the 2010 California pertussis outbreak (21), (iii) 385 surveillance isolates collected between 2010 and 2012, and (iv) 216 isolates from the 2012 Washington pertussis outbreak (2). Historical isolates were received by the public health laboratories of 46 states between 1935 and 2009. They were selected for analysis using random sampling stratified by geography and time to ensure as much equal geographic and temporal representation as possible (12). Very little to no clinical information is available for the majority of the historical isolates. The 2010 California outbreak isolates were collected between February 2010 and November 2010. The routine surveillance isolates were all isolates collected between January 2010 and November 2012 from six states participating in Enhanced Pertussis Surveillance (Colorado, Connecticut, Minnesota, New Mexico, New York, and Oregon) and from other state public health laboratories. The Enhanced Pertussis Surveillance is supported by the CDC Emerging Infection Program network to conduct enhanced pertussis surveillance. The Washington 2012 isolates comprised all isolates received from the Washington Public Health Department and the Seattle Children's Hospital, collected between May 2011 and October 2012.

**PCR screening.** Conventional PCR screening for the IS*481* insertion within *prn* was performed at the CDC with the PF and PR primers from Mooi et al. (22), following methods previously described (12) (Table 1). These primers are just internal to the 5′/3′ ends of the *prn* gene and produce a 2.5-kb amplicon. Further combinations of primers, such as AF, AR, BF, and BR from Mooi et al. (22), Prn-F and Prn-R from Otsuka et al. (19), and newly constructed primers to encompass the entire gene and promoter region, were also utilized (Table 1). At the CDC, PCRs were run as follows: in a 50-μl reaction, 2.7 U of Roche Diagnostics Expand high-fidelity enzyme (Indianapolis, IN) was used with final concentrations of 1.5 mM $MgCl_2$ buffer, 2 μM forward/reverse primers, 0.2 μM deoxyribonucleotide triphosphates (dNTPs), and 10% dimethyl sulfoxide (DMSO). Cycling conditions were 95°C for 15 min, 20 cycles of 94°C for 45 s, 55°C for 45 s, and 72°C for 2.5 min, and 10 cycles of 94°C for 45 s, 50°C for 45 s, and 72°C for 2.5 min. Isolates were identified as either *prn*IS*481* negative for producing the expected 2.5-kb amplicon or as *prn*IS*481* positive for producing a 3.5-kb amplicon that contains the IS*481* insertion.

**Western blot analysis.** After PCR screening, 100 isolates were selected for characterization by Western blotting. Because isolates harboring the *prn*IS*481* insertion have been shown to no longer express pertactin (13, 16, 19), more emphasis was placed on selecting the *prn*IS*481*-negative isolates for detection of pertactin deficiency due to other types of possible mutations. The selection of isolates was randomized following certain criteria to ensure an equal representation between the four groups of isolates, as was possible: historical 1994-2009 (the first *prn*IS*481*-positive isolate was identified in 1994), California 2010, U.S. 2010-2012, and Washington 2012. The selection criteria were (i) 25 isolates from each group, (ii) 91 *prn*IS*481*-negative isolates, (iii) 9 *prn*IS*481*-positive isolates to confirm the lack of protein expression, and (iv) random selection of both the *prn*IS*481*-positive and *prn*IS*481*-negative isolates from each of the four groups (8 subgroups total) except for the sole 1 and 2 *prn*IS*481*-positive isolates found in the historical and California 2010 group, respectively (Fig. 1).

For Western blot analysis, the cultures were grown overnight at 35°C in Stainer-Scholte medium. The lysates were made by processing harvested cells in saline using a FastPrep homogenizer. Approximately 5 μg of protein was run on 12% NuPAGE gels in morpholinepropanesulfonic acid (MOPS) buffer (Life Sciences, Carlsbad, CA) and transferred to polyvinylidene difluoride (PVDF) membranes using an iBlot apparatus. Pertactin was detected on Western blots using the α-goat WesternBreeze kit (Life Sciences) with National Institute for Biological Standards and Control (NIBSC) anti-69K antiserum 97/558 at a 1:1,000 dilution. The WHO strain 18323 served as the pertactin-positive control.

**Sequencing analysis.** The *prn* alleles of a total of 93 isolates were sequenced (Fig. 1). These included the 18 pertactin-deficient isolates found through the Western blot analysis. These 18 *prn* alleles were sequenced by ACGT (Wheeling, IL) using primers that covered the entire coding region (23). The remaining 75 isolates were from the Washington 2012 outbreak group. Of these 75 isolates, 10 were *prn*IS*481*-positive isolates to confirm the PCR screening results and the presence of the insertion. The other 65 isolates were the remaining *prn*IS*481*-negative isolates from the Washington 2012 outbreak that were not selected for the Western blot analysis. The 75 isolates were sequenced at the CDC using Applied Biosystems 3130xl analyzers (Foster City, CA) with a previously described method (24) and analyzed using DNAStar Lasergene 9 (Madison, WI).

The sequence identity was based on the Tohama I sequence (GenBank accession number NC_002929.2), and alignment with IS*481* was performed with GenBank accession number M22031 (25).

**Pulsed-field gel electrophoresis.** Pulsed-field gel electrophoresis (PFGE) was performed on all 1,300 isolates at the CDC with a method previously described (26). Briefly, the DNA in agarose plugs was digested with restriction enzyme XbaI. The restricted plugs were placed in 1% agarose gels. The electrophoresis was conducted in 0.5× Tris-borate-EDTA buffer at 6 V/cm for 18 h at 14°C with a ramped switch time of 2.2 to 35 s. The gels were stained with ethidium bromide, and the DNA bands were visualized using UV light. A digital image in the TIFF format was captured for each gel. The TIFF images were analyzed using BioNumerics software, version 5.01 (Applied Maths, Inc., Austin, TX). Profiles consisted of bands in the range of 125 to 525 kb.

**Gene sequence accession numbers.** Five novel sequences were identified and submitted to GenBank: KF804023 for the *prn*IS*481* insertion at nt 2735, KF804024 for the stop mutation at nt 760, KF804025 for the nucleotide G insertion at nt 1180, KF804026 for the 5′ deletion at nt −2090-478, and KF804027 for the rearrangement in the promoter region.

## RESULTS

Of the 1,300 isolates screened by *prn* PCR and/or later identified by Western blotting and sequence analyses, 276 (21%) were identified as *prn*IS*481* positive (Fig. 1 and 2). Strikingly, only one (0.2%) *prn*IS*481*-positive isolate was found in the historic collec-

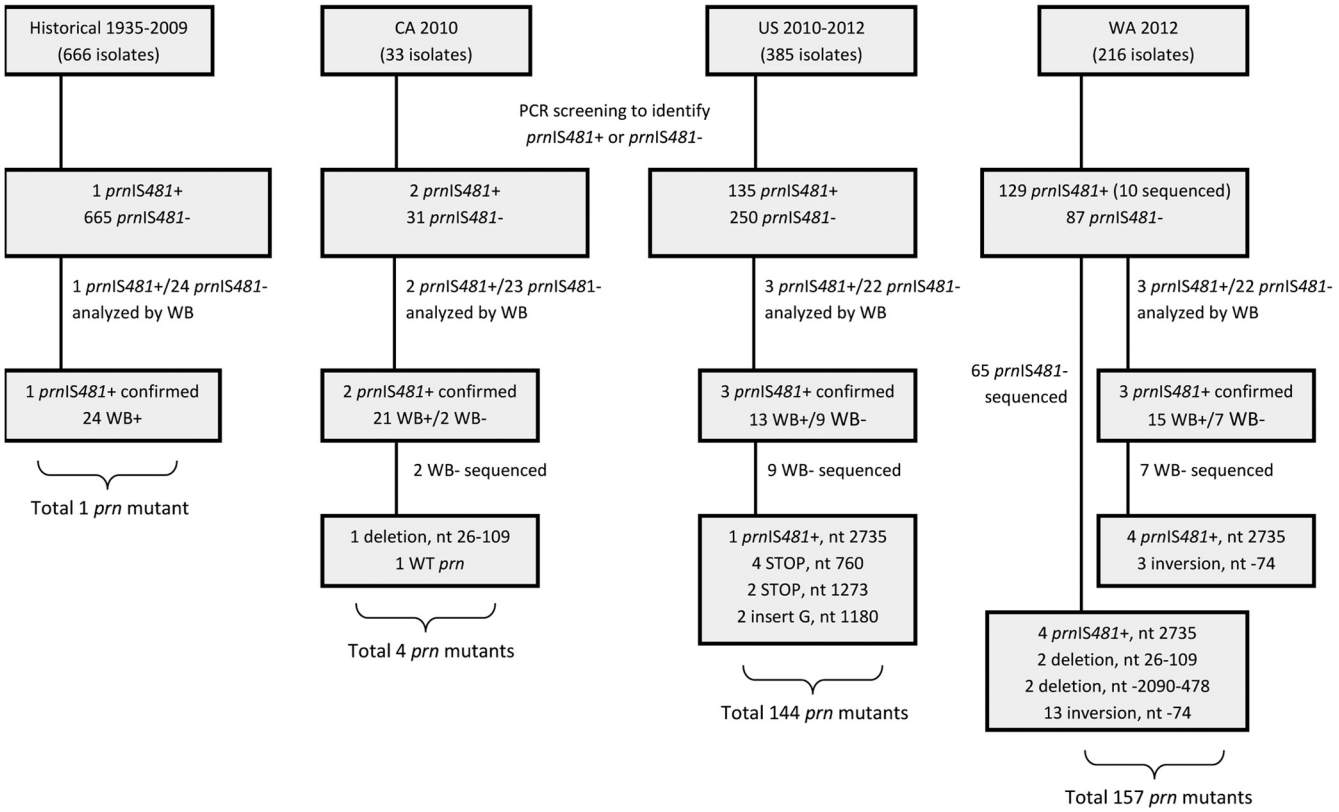


**FIG 1** Flow chart of the progression of pertactin deficiency identification, from the conventional PCR screening of the IS*481* insertion in the *prn* gene to Western blot (WB) analysis and sequencing. IS*481* positive, IS*481* insertion in *prn*; IS*481* negative, IS*481* insertion in *prn* not detected; WB+, pertactin protein present by Western blotting; WB−, pertactin protein absent by Western blotting; WT, wild type; STOP, stop codon.

tion that dated from 1935 to 2009 (Fig. 2A). This isolate was collected in 1994 from North Carolina, while the next *prn*IS*481*-positive isolates were observed in 2010 in both the California outbreak and in the U.S. 2010-2012 collection (Fig. 2A). In the California 2010 outbreak, only 2/33 (6%) *prn*IS*481*-positive isolates were identified. In the Washington 2012 outbreak, more than half (63%) of the isolates collected were *prn*IS*481* positive (137/216 isolates). This observation does not appear to be a regional or isolated incidence, as the U.S. isolates captured from 2010 to 2012, when stratified by year, also show a similar trend, with 14%, 40%, and 53% of the isolates being *prn*IS*481* positive for the years 2010, 2011, and 2012, respectively (Fig. 2B).

The Western blot analysis of 100 selected isolates confirmed that the nine *prn*IS*481*-positive isolates did not express pertactin, and of the 91 *prn*IS*481*-negative isolates, 18 were deficient for pertactin protein expression (Fig. 1). Sequence analysis of these 18 isolates revealed seven different mutations in *prn* (Fig. 1). Furthermore, sequencing of the remaining 65 *prn*IS*481*-negative isolates from the Washington 2012 outbreak revealed an additional 21 mutants.

In total, 306 pertactin-deficient isolates were detected. The geographic spectrum of these 306 pertactin-deficient isolates was far-reaching, with mutations found in Arizona, California, Colorado, Connecticut, Florida, Georgia, Massachusetts, Minnesota, Missouri, North Carolina, New Mexico, New York, Oregon, Pennsylvania, Vermont, and Washington. The highest percentage (12.1%) was in Washington, likely due to its having the largest, most recent collection of isolates submitted, and all *prn*IS*481*-negative isolates were sequenced. The next highest proportions were observed in Oregon (4.4%), Minnesota, New York (1.5%), and Massachusetts (1%).

From the 306 pertactin-deficient isolates, 10 different types of mutations in the pertactin coding region were identified, including two deletions, single nucleotide substitutions resulting in a stop codon, and single nucleotide insertions resulting in frameshift mutations (Table 2; Fig. 3). The majority of the mutant types (9/10) revealed *prn2* alleles. The sequencing analysis of the *prn*IS*481* insertion revealed that the insertion was positioned in various locations throughout the gene (Table 2). Two of these insertion mutations were previously identified in the United States (GenBank accession numbers KC445198 and KC445197), with the insertion of the IS*481* in either direction (20). A third mutation, a truncated pertactin protein with the stop codon at amino acid (aa) 425, was also identified from the United States (GenBank accession number KC445199) (20). Only three isolates were identified as having the *prn1* alleles, and they contained a 5′ signal sequence deletion, a mutation that was previously identified in Japan (GenBank accession number AB670735) (19). Interestingly, nine *prn*IS*481*-positive isolates had the IS*481* insertion at the 3′ end of the gene (nt 2735), which was outside the coding region amplified in the initial PCR screen (Fig. 1). Of note, the predicted protein resulting from this IS*481* insertion is nearly full length in size, yet this mutation resulted in a lack of detectable pertactin expression by Western blotting. PCR and sequencing of

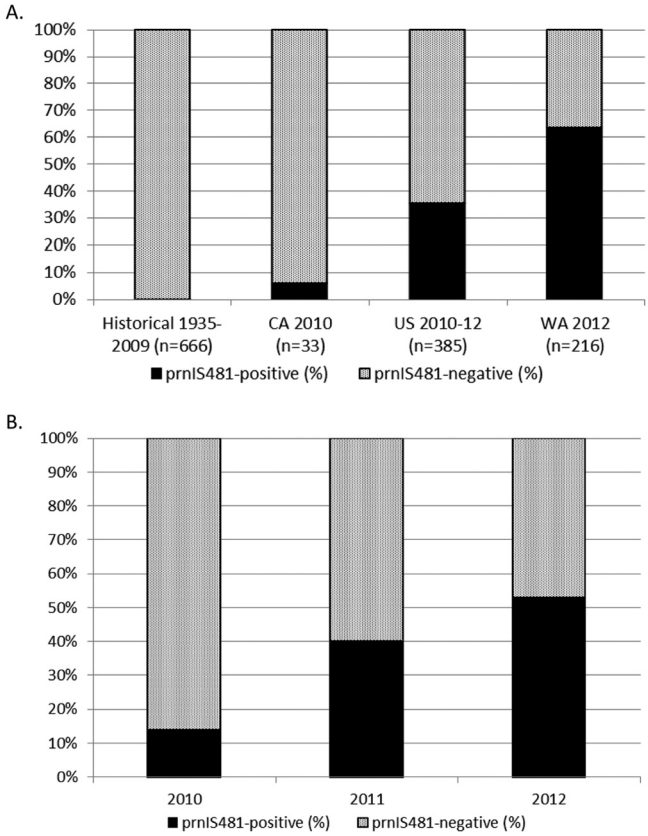


**FIG 2** (A) Proportion of *prn*IS*481*-positive *B. pertussis* isolates, identified by PCR and sequencing, from the 2012 Washington outbreak, 2010-2012 U.S. collection, 2010 California outbreak, and 1935-2009 U.S. historic collection, stratified by origin and time period. (B) Proportion of *prn*IS*481*-positive *B. pertussis* isolates from the U.S. 2010-2012 collection, stratified by year.

the region upstream of the coding sequence revealed a large deletion that encompassed the promoter and 5′ region (Table 2, type VIII) and a genomic rearrangement that disrupted the pertactin promoter (Table 2, type IX). Since the type VIII mutation was found only through the sequencing of the Washington 2012 *prn*IS*481*-negative isolates, it was later confirmed to lack protein expression through Western blot analysis. Finally, one isolate had a wild-type promoter and coding region, suggesting that it contains a mutation in the regulatory system controlling pertactin expression (Table 2, type X).

The predominant PFGE profiles of the 994 isolates that did not have a mutation identified (pertactin present) and the 306 isolates that were identified as lacking pertactin protein (pertactin deficient) are shown in Fig. 4. CDC 013, a profile that has predominated in the United States since 1999, reaching a peak at 48% in 2001 and gradually decreasing since the peak (27), was a predominant profile in the pertactin-present isolates of all four time periods, representing 20% (203/994) of the pertactin-present isolates but only 5% (16/306) of the pertactin-deficient isolates. Two profiles that have recently emerged, CDC 237 and CDC 253, predominated in only the pertactin-deficient groups. Finally, unlike the pertactin-present isolates, two profiles, CDC 002 and CDC 237, represented 55% (167/306) of the identified pertactin-deficient isolates from 2010 to the present. Interestingly, while CDC 237 is a much more recently identified profile, CDC 002 was identified in the 1980s. CDC 002 accounted for only 4% of U.S. isolates collected between 2000 and 2009 and increased to 9%, 24%, and 28% of isolates from 2010, 2011, and 2012, respectively (26, 28).

## DISCUSSION

This work marks the first documentation of the frequency and geographic distribution of pertactin-deficient isolates in the United States. Based upon data from PCR, Western blot analysis, and sequencing, pertactin-deficient isolates have been found na-

**TABLE 2** Characterization of pertactin-deficient isolates by sequencing analyses

| Mutation type[a] | Pertactin mutation, nucleotide no. | Pertactin MLST[b] allele | Predicted pertactin protein[c] | State (no. of isolates) | Year (no. of isolates) | PFGE[d] (no. of isolates) |
|---|---|---|---|---|---|---|
| I | IS*481*, 246[e] | *prn2* | Stop at aa 87[f] | WA (2) | 2011 (1) 2012 (1) | CDC 253 (2) |
| II | IS*481*, 1613[e] | *prn2* | Stop at aa 543 | WA (8) | 2012 (8) | CDC 237 (4) CDC 013 (2) CDC 010 (1) CDC 002 (1) |
| III | IS*481*, 2735[e] | *prn2* | Stop at aa 913 | WA (8) | 2012 (7) | CDC 322 (6) CDC 002 (1) |
| | | | | | 2011 (1) | CDC 046 (1) |
| | | | | NY (1) | 2012 (1) | CDC 046 (1) |
| IV | Stop, 760 | *prn2* | Stop at aa 254 | NY (4) | 2011 (1) 2012 (3) | CDC 242 (4) |
| V | Stop, 1273 | *prn2* | Stop at aa 425 | VT (1) | 2011 (1) | CDC 002 (1) |
| | | | | NY (1) | 2012 (1) | CDC 002 (1) |
| VI | Insert G, 1185 | *prn2* | Frameshift at aa 395 | MN (2) | 2012 (2) | CDC 002 (2) |
| VII | Deletion, 26–109 | *prn1* | Deletion of 28 aa | CA (1) | 2010 (1) | CDC 268 (1) |
| | | | | WA (2) | 2012 (2) | CDC 260 (2) |
| VIII | Deletion, −2090 to 478 | *prn2* | Deletion of 2.6 kb | WA (2) | 2012 (2) | CDC 013 (1) CDC 046 (1) |
| IX | Inversion, −74 | *prn2* | Inversion of ~22 kb in promoter | WA (16) | 2011 (2) | CDC 010 (2) |
| | | | | | 2012 (14) | CDC 010 (12) CDC 002 (2) |
| X | Wild type | *prn2* | Full-length 915 aa | CA (1) | 2010 (1) | CDC 013 (1) |

[a] At least one isolate of each mutation type has been confirmed by Western blot analysis to lack protein expression.
[b] MLST, multilocus sequence typing.
[c] *prn* lies in the region between nt 1,098,091 and 1,100,823 of the Tohama I complete genome (GenBank accession no. NC_002929.2).
[d] PFGE, pulsed-field gel electrophoresis.
[e] Shows forward IS*481* insertion as example, according to GenBank accession no. M22031.
[f] Stop, stop codon; aa, amino acid.

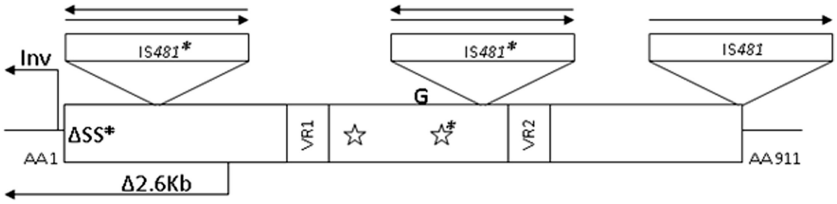


**FIG 3** Location of mutations in the *prn* gene, identified through PCR, Western blotting, and sequencing analyses. Mutations included 3 IS*481* insertions, 2 premature stop codons (☆), a nucleotide insertion (G), a signal sequence deletion (ΔSS), a large deletion in the 5′ region and upstream (Δ2.6 kb), and a large inversion of the promoter region (Inv). IS*481* insertions were found in both directions, where indicated, according to alignment with GenBank accession number M22031. Mutations marked with an asterisk (*) were identified previously. AA, amino acid; VR, variable region.

tionwide. While the first pertactin-deficient isolate was identified here in 1994, the high frequency appears to be more recent with the expansion of pertactin-deficient isolates first observed in 2010. It must be noted that this work was based on an initial screening method that did not encompass all the possible mechanisms for losing pertactin protein expression. Likewise, the possible existence of pertactin molecules that can be expressed but are not functional cannot be discounted. Therefore, while the subset of isolates analyzed by Western blotting was a good representation of each of the four time period groups, the current numbers of each type of mutation may be underrepresented. Finally, because one protein-deficient isolate with a wild-type *prn* (Table 2) was identified, the sequencing of the coding region may not be sufficient to determine the lack of protein expression.

The loss of pertactin expression has been reported from several other countries (13, 16–19). Despite the emergence of pertactin-deficient isolates occurring over a decade ago, the expansion of these isolates has been a recent phenomenon in the United States. The identification of our first *prn*IS*481*-positive isolate in 1994 is similar to discoveries from other countries, namely, in Italy, where a mutant was identified from isolates collected between 1993 and 1995 (18), and in Japan, where the earliest isolate from a collection between 1990 and 2009 was identified in 1997 (19). In other countries, pertactin-deficient isolates were analyzed in more recent collections, such as 2000-2011 for France and 2006-2011 in Finland (13, 16, 17). Not only has the expansion been recent in the United States but also the proportion of pertactin-deficient isolates in the circulating population is noteworthy, with >50% of the isolates received by the CDC in 2012 being pertactin-deficient isolates. In France, the highest prevalence was 13% in 2011, and in Finland, only 2 of 76 isolates were found to be pertactin deficient (13, 16, 17). The variety of mutations has also differed between countries, with only two mutation types, the IS*481* insertion in one location and the 5′ signal sequence deletion, identified in the Japanese collection and several more in the French collection, including a large deletion of the promoter and 5′ region (different from our large-deletion mutants), IS*481* insertions, and stop codon mutations (13, 17, 19).

Indeed, the fact that *B. pertussis* has eliminated pertactin expression using a variety of mutational events indicates strong selection for loss of pertactin. Clonal expansion of specific mutations does appear to be occurring in multiple states, regardless of whether it was from an outbreak population or a surveillance site (Table 2). Two PFGE types, CDC 002 and CDC 237, also appear to predominate among the recent pertactin-deficient isolates (Fig. 4). However, the lack of a coassortment of the mutations causing a pertactin deficiency with specific PFGE patterns suggests that these mutations are perhaps independent of pertussis clonal selection and redistribution or at least fall under selective pressures different from those of the whole genome (Fig. 3). Additionally, it

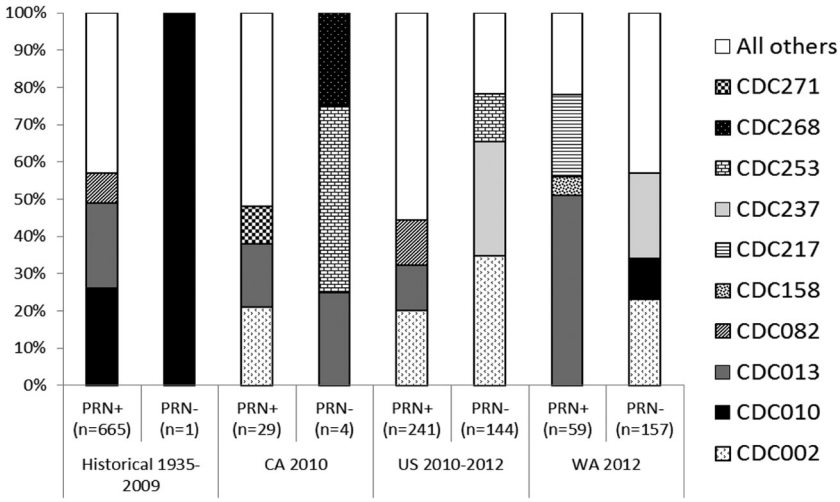


**FIG 4** PFGE profiles of the 1,300 *B. pertussis* isolates analyzed for pertactin deficiency, stratified by origin and time period. The top three predominant profiles are indicated per group; all remaining profiles are identified as "All others." PRN+, *B. pertussis* isolates that do not contain genetic mutations and/or express pertactin; PRN−, *B. pertussis* isolates that are pertactin deficient as tested by PCR screening, Western blot analysis, and *prn* sequencing; *n*, number of isolates.

must be noted that not all the *prn*IS*481*-positive isolates were sequenced, and these mutation types make up the majority of the total population of mutants, so the clonal nature of these mutations remains unclear.

The presence of genetic mutations in one of the vaccine antigens reveals the importance of remaining vigilant in monitoring the emergence of mutations in other vaccine antigens or those targets used for molecular diagnostic testing. Indeed, the deletion of the pertussis toxin gene was observed once in France in 2009 (17). However, *prn* is not a target commonly used for pertussis diagnosis through PCR. Additionally, the CDC Pertussis and Diphtheria Laboratory currently monitors multiple PCR targets in their isolate and specimen collections and, to date, no mutations in these diagnostic targets (29) have been observed.

Research is shedding more light on the function of pertactin in virulence and pathogenesis, revealing multiple and/or potentially conflicting roles. One pertactin-deficient isolate showed improved entry into human monocyte-driven dendritic cells, suggesting that pertactin may play a role in preventing internalization (30). Earlier opsonophagocytic work supports this suggestion, finding that anti-pertactin antibodies were crucial for *B. pertussis* phagocytosis (31). However, cytotoxicity remains high despite the loss of pertactin, suggesting that some functional redundancy may be involved (17); indeed, the identification of numerous autotransporters, with as of yet undefined roles, has been suggested to be the reason for the comparable virulence in pertactin-deficient isolates (32). From a clinical perspective, the loss of pertactin does not appear to alter disease severity, because a recent study of hospitalized infants <6 months old showed that pertussis disease, measured by the presence of classical symptoms, such as apnea, vomiting, paroxysmal cough, and whoop and caused by pertactin-positive or pertactin-deficient isolates, had a similar clinical course (33). The mechanism of the selective advantage leading to the expansion of pertactin-deficient variants still remains unclear.

Previous hypotheses suggested that the acellular vaccine helped drive the expansion of these particular clones, as the bacterial genome was already well adapted to the environment and only needed small mutations to thrive in current vaccinated populations (13, 15). However, vaccination with the acellular vaccine may not be the sole cause of this recent phenomenon in the United States as the first pertactin-deficient isolate discovered was from the time of early implementation of the acellular vaccine primary series, suggesting that these isolates may have already been in circulation. It is interesting to note that the phenomenon coincides with the time when the United States is experiencing large outbreaks in a population receiving primarily acellular vaccine (2, 3); however, pertactin-deficient isolates have expanded only within the last few years despite more than a decade of acellular vaccine usage.

Regardless of the reasons for this recent increase in pertactin-deficient isolates in the United States, clinical information, *in vivo* functional assays, animal modeling, and vaccine effectiveness studies are crucial not only for understanding the clinical implications of these changes but also for helping to develop and test new vaccine formulations (34). Vigilant monitoring of the cellular and molecular changes of not only *B. pertussis* but also other *Bordetella* spp., via bacterial isolation and molecular methods, is critical for determining the next steps for protecting the health of the public against these highly contagious pathogens.

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